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ABSTRACTS FROM THE ORIGINAL PAPERS.

A New Method for the Isolation of Diamines.

By Masakazu YAMADA.

(Received Feb. 3rd., 1926)

Recently A. Kossel and K. E. Gross, proposed a new method for the isolation of arginine with naphthol yellow. (Z. Physiol. Chem., 135, 167–174, 1924) The auther applied the method to Saké and Shōyu and could obtain much crystalline precipitate in the latter, though none in the former. This precipitate was easily recrystallized from boiling water in beautiful rosettes, which were found to be the mixture of the double salts of putrescine and cadaverine with the naphtol yellow.

As it was suspected that the precipitation in the case of Shōyu might be due to "salting out", caused by the common salt which is present generally in an amount of 20 % in it, more salt was added to make the precipitation complete. Thus, the auther's modified method for separating organic bases with naphthol yellow is carried out as follows:–

To the solution containing organic bases, for instance Saké, is added a large quantity of hot saturated solution of naphthol yellow and the mixture is shaken vigorously. After twenty four hours' standing, the precipitates, if they are formed as in the case of arginine, are filtered and may be investigated.

If not, the precipitation may be effected by the salting out with common salt. Then the precipitates are filtered off by suction and thoroughly washed with saturated salt solution. After that, they are treated in the same way as was given by Kossel and Dross: namely the precipitates are decomposed by dissolving them in sulphuric acid (33 volume per cent) and the coloring matter, which crystallizes out on cooling, is filter off. The process is repeated again. Both the filtrates are mixed and decolorized with animal charcoal. Sulphuric acid is then removed by baryta and excess of the latter is removed by carbon dioxide. The The carbonates of the bases then can be easily converted into convenient derivatives like picrate etc.

Ammonium-sulphate also salts out the double salts of the coloring matter with bases, but magnesium sulphate has no such property.

As a rule, the auther does not use basic lead acetate or other reagents to remove albuminous matter from the liquid, to which the naphtol yellow was to be added. It might be suspected that this abridgment might cause too high value for the bases. That, in reality, it is not the case, can be deduced from the following comparative test with Shōyu.

Total nitrogen precipitated by naphthol yellow in 100c.c. of Shōyu without preliminary treatment of basic lead acetate: 0.02388 g.

Total nitrogen precipitated by naphthol yellow in 100 c.c. of Shōyu with preliminary treatment of basic lead acete: 0.02236 g.

The nophtol yellow method seems to be very suitable for the separation of diamines like putrescine and cadaverine as well as arginine, but as regards the other bases, the further experimemts are in dispensable to reach some conclusion. The results of actual separation from solutions containing bases are compared in the following table.

P. T.: Bases precipitated with phosphotungstic acid

N. Y.: 〃 〃 〃 Naphthol yellow by the auther.

Substance		Reagent	Amount of Substance obserbed	Bases iso'ated	Observer
Shōyu		P. T.	2 L.	Newbase $C_6H_9N_3$ 1g. Lysine 2.6g. $C_4H_{12}N_2$ 0.2g.	Suzuki, Asō, Mitarai, 1907
		N. Y.	2 L.	Putrescine picrate 0.16g. Cadaverine picrate 0.4g.	1925
Tamari Shōyu		P. T.	2 L.	$C_6H_9N_3$ 1.4g. Putrescine 0.6g. Ornithine 1.4g.	Yoshimura 1909
		N. Y.	5 L.	Putrescine picrate 3.96g. Cadaverine picrate 2.42g.	1925
Miso	Hatchō-miso	P. T.	1 kg.	Lysine hydrochloride 9.6 g.	Takahashi, Shinohe 1908
		N. Y.	500 g.	Cadaverine picrate 1.18 g.	1925
	Inaka-miso	N. Y.	500 g.	Cadaverine picrate 1.18 g.	1925

Sendai-miso	N. Y.	500 g.	Cadaverine picrate 9.28 g.	1925
Nattō*	P. T.	6.8 kg.	Guanine, Xanthine, Hypoxanthine	Yabe, 1894
	P. T.	4 kg.	Xanthine, Hypoxanthine and a base-Its Gold-salt melts at 118-120°C and containes Au 41.42%, Picrate melts at 193-144°C	Itō, 1925
	N. Y.	1 kg.	Cadaverine picrate 1.08 g,	1925
Putrid Saké	P. T.	18 L.	Choline picrate 2.5 g. Histamine pic. 0.52g. Cadaverine pic. 0.14 g.	Kurono, 1917
	N. Y.	5 L.	Codaverine picrate 0.37 g.	1925
Saké	P. T.	10 L.	Lysine picrate 1.1 g. (F. 230°C)	Takakashi 1913
	P. T.	25 L.	Lysine picrate 3.8 g. Histamine pic. 0.15 g. Lysine pic. 0.5 g.	Kurono 1917
	N. Y.	5 L.	Cadaverine picrate 0.65 g.	1925

* Nattō is a steamed soy-bean upon which Bacillus Nattō from the straw has been developed

Studies on Enzyme II.

The influence of glycocoll upon the urease action.

By Matsunosuke KITAGAWA.

(Received Jan. 11th, 1926)

The favourable influence of glycocoll upon the soy-bean urease action is well known, but mechanism has not yet been established. The soy urease used in the present experiments was purified by adsorption method or dialysis in order to remove proteins and diffusable substances as much as possible.

The couslusions from my experiments are as follows :-

(1) That the glycocoll has no influence upon the action of a crude urease preparation is due to the existence of a pretty amount of proteins and diffusable substances in the preparation.

(2) The less the quantity of proteins and some diffusable substances in reaction system, the greater is the decrease of activity of the purified urease in

short time reaction and the reduced activity is recovered to the normal by the addition of glycocoll.

(3) The phosphate buffer mixtures commonly used in urease reaction are injurious, but their injurious action is counteracted by the addition of glycocoll. The buffer mixtures destroy partly the urease.

(4) Above a certain degree of concentration urea gives the same inhibitory action as phosphate upon urease. This inhibitory action is also counteracted by the addition of glycocoll. The influence of temperature, below 35°C, is not recognized in such a short time-reaction.

(5) The aqueous solution of urease becomes gradually inactive on keeping a long time. This decrease in activity is partly avoided by means of protein or glysocoll. The decrease of activity in a short time-reaction is, however, not due to the destruction of enzyme.

(6) In the case of crude urease alone or of purified urease with glycocoll, the enzymatic activity is exactly proportional to its concentration to a large extent.

(7) After all the restorations of diminished activity of purified urease by means of glycocoll seem to be due neither to the protection of enzyme from destruction, nor to the reactivation of inactive enzyme complex, nor to the occurence of a labile compound between urea and glycocoll. They seem to be due to the fact that some unfavourable changes of colloidal nature of urease system with insufficient quantity of protein etc. are reversibly restored to a favourable condition by the presence of some glycocoll.

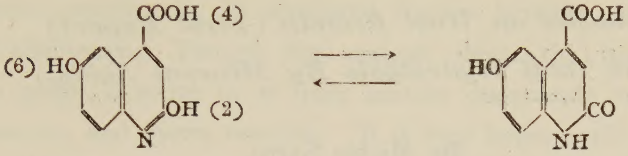
On the chemical Constitution of β-Acid (Dioxyquinoline-carbonic acid) obtained from Suzuki's Crude Oryzanin by Hydrolysis.

Part III.

By Yoshikazu SAHASHI.

(Received Feb. 2nd. 1926)

In the preceding paper, the author has shown that β-acid will very likely have the following formula from the result of his several analytical experiments :

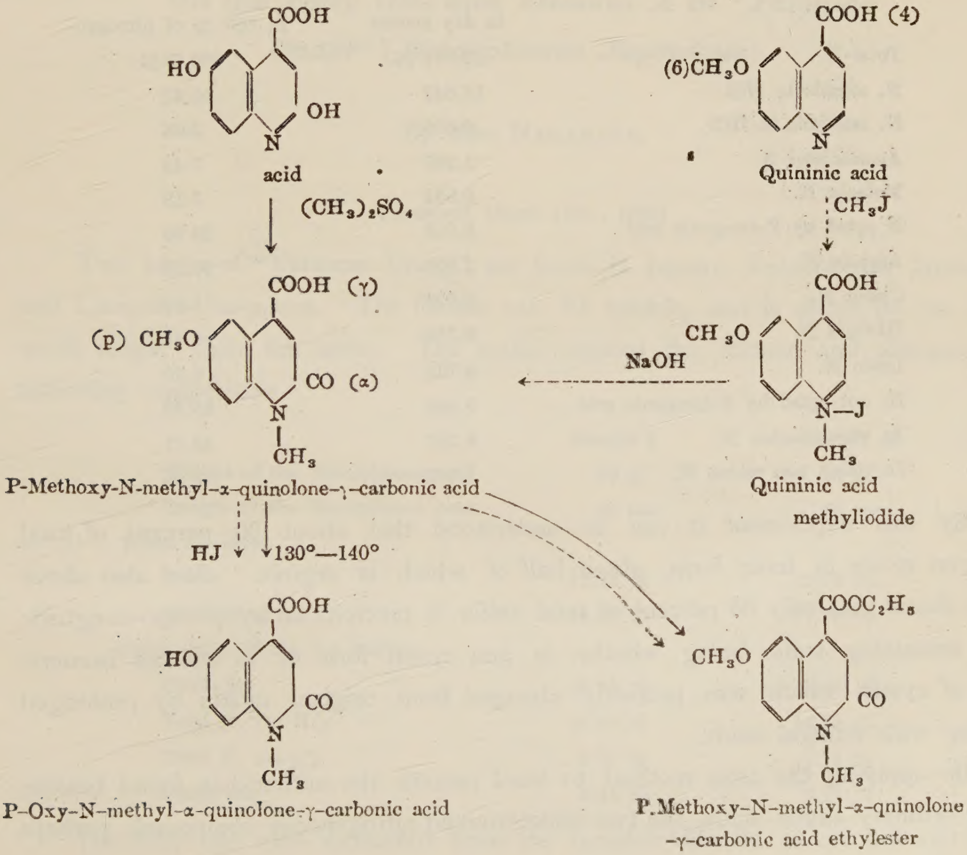


2.6-Dioxyquinoline-4. carbonic acid

Taurometic form

But as to the synthetical researches, it was left under his further investigations.

In 1894 Roser prepared *p*-methoxy-N-methyl-*α*-quinolone-*γ*-carbonic acid from quininic acid (A. 282 367). So the author synthesized this acid by the Roser's method, and found that its constitution is exactly same to that of the acid obtained from *β*-acid by treating with $(CH_3)_2SO_4$. Moreover he ascertained this fact by obtaining just the same ethylester from both *β*-acid and quininic acid as shown in the following diagram. Thus the author confirmed that his supositional formula for *β*-acid is the right one.


COOH
HO
OH
N
acid
(CH₃)₂SO₄
COOH (γ)
(p) CH₃O
CO (α)
N
CH₃
P-Methoxy-N-methyl-α-quinolone-γ-carbonic acid
HJ
130°—140°
COOH
HO
CO
N
CH₃
P-Oxy-N-methyl-α-quinolone-γ-carbonic acid
COOH (4)
(6)CH₃O
N
Quininic acid
CH₃J
COOH
CH₃O
NaOH
N—J
CH₃
Quininic acid methyliodide
COOC₂H₅
CH₃O
CO
N
CH₃
P.Methoxy-N-methyl-α-qninolone -γ-carbonic acid ethylester

Studies on Wool Protein (First Report)
(The Total Hydrolysis By Mineral Acids.)

By Michio Saito.

(Received Dec. 16th. 1925)

The author has investigated the amion–acid distribution of sheep's wool according to both Van Slyke's method and Emil Fischer's ester method. The wool used in this experiment was taken from sheep of Merino cross–breed and purified repeatedly with hot ether and water. The elementary analysis showed following result (in dry matter) :–

C	H	N	S	O
48.98	6.66	16.97	3.03	24.36

The nitrogen distribution of wool protein is as follows :–

	in dry matter	in 100 % of nitrogen
Total–N	16.974 %	100.00%
N. soluble in HCl	16.349	96.32
N. insoluble in HCl	0.624	3.68
Ammoniacal N.	1.265	7.45
Melanin N.	0.541	3.19
N ppted by P–tungustic acid	5.048	29.95
Arginin N.	3.003	17.70
Cystin N.	0.525	3.09
Histidin N.	0.755	4.45
Lysin N.	0.798	4.70
N. not ppted by P–tungustic acid	9.460	55.73
In which amino N.	8.227	48.47
In which non amino N.	1.232	7.26

By this experiment it can be understood that about 30 percent of total nitrogen exists in basic form, about half of which is arginin. And also above result shows that only 35 percent of total sulfur is precipitated by phospo–tungustic acid, remaining sulfur being whether in non cystin form or in another isomeric form of cystin which was probably changed from original cystin by prolonged heating with mineral acids.

By applying the ester method to wool protein the author has found besides many ordinary amino–acids, the two undetermined nitrogeneous compounds perhaps both the same amino-acid, in distillated fracton between 105–180°C. and in residues of distillation. Though the analysis shows the ressemblance of this substance to prolin, it seems to be quite another compound; because it shows no ninhydrin reaction and pyrrol reaction. It is very hygroscopic and produces the copper salt of green colour with $Cu(CH)_2$. This substance will be studied in further experiment.

Amino-acids determined by author's experiment are as follows :–

glycocoll	0.35	alanin	7.0
valin	3.8	leucin	12.5
phenyl alanin	5.2	glutamic acid	9.1
asparatic acid	1.4	tyrosin	4.6
arginin	9.3	histidin	4.1
lysin	2.7	sulfur	3.03

On the Fatty Oils and Vitamin A in "Yatsume Unagi" (Entosphenus Japonicus).

By Ziro NAKAMIYA.

(Received March 11th., 1926)

Two kinds of "Yatsume Unagi" are found in Japan:– Entosphenus Japonicus and Lampetra Japonicus. The former can be eatable, and is about 50 cm. long much longer than the latter. The author studied the former and obtained the following composition.

	Sample I	Sample II
Weight of the Entospheuns used	60 g.	48 g.
Length of the Entospheuns used	49 cm.	46 cm.
When air-dried		
Water	11.85 %	12.72 %
Dry matter	88.15 %	87.28 %
Composition of the dry matter		
Crude fat	55.55 %	43.90 %
Total N. (as N.)	15.66 %	14.90 %
Total P. (as p.)	0.88 %	1.71 %
Crude ash	5.44 %	5.44 %

The fatty oils were extracted from the samples with 94 % alcohol and then with ether for several times; the yield was more than 46 % of the air-dried samples. The oils were dark brown, and had a special odour, solidifying in the winter season.

The general properties of the fatty oils were as follows:

Specific gravity	0.920 (23.5°C)
Solidifying point of the mixed fatty acids	23.9°C
Refractive index	1.435 (Abbe's, 40°C)
Iodine value	13.01
Saponification value	173.5
Acetyl value	31.0
Acid value	84.2
Insoluble bromide value (%)	6.4
Unsaponifiable mater (%)	2.7

The general charactor of the mixed fatty acids obtained from the dark brown oils above by saponification was as given below.

Specific gravity	0.859 (15.5°C)
Melting point	33.7°C
Solidifying point	23.9°C
Refractive index	1.462 (Abbe's, 40°C)
Iodine value	159.4
Neutralisation value	191.1
Mean molecular weight	293.4

By the lead salt ether method, the liquid unsaturated fatty acids were obtained; the yield being 75.2 %. Then, by Hazcra's method the fatty oils were oxidized with alkaline $KMnO_4$ solution, and thus white powder was precipetated and purified by means of alcohol. It was separated into the ether soluble part and the ether difficulty soluble part and the analyses of these two parts were as below.

Sample		M. P	C %	H %
Ether soluble part	I	109°C	68.01	11.18
	II	108°C	68.02	10.58
Ether difficultly soluble part	I	88°C	68.80	11.16
		88°C	69.35	11.06
	II	118°C	67.41	10.92
	III	125°C	68.10	10.94
	IV	125°C	68.04	11.23
Calculated as $C_{18}H_{36}O_4$			68.35	11.40

Therefore the author considered that a great part of the fatty oils must be

"Dioxystearic acid" (Gröger 125°C; Saytzeff 136.5C) being mixed with some dioxystearic acid (isomer, m. p. 99.5°), which is derived from elaidic acid, etc.

The solid saturated acid that separated from the mixed fatty acids above was white flowerlike crystalline, having melting point of 52.5–53.5°C, dissolving easily in absolute alcohol, ether, benzol and chloroform, but being insoluble in water. From these facts, the author concluded that it is "Myristic acid" $C_{14}H_{28}O_2$ (53.8°C)

Analysis :-	C %	H %
Sample I	73.77	12.30
II	74.32	12.39
Calculated	73.59	12.25

Vitamin A was extracted from the dark brown oils by K. Takahashi's method. The yield of the crude "Biosterin" obtained was 0.3–0.4%, and its refractive index was 1.508 at 30°C. The purified substauce contained 78.30 % carbon and 11.37 hydrogen.

A comparison of the composition of vitamin A was made as follows:

	C %	H %
Vitamin A from cod liver oil	81.07	11.14 (ave.)
Calculated $C_{27}H_{44}O_2$	81.00	11.00
Vitamin A from "Aonori" (Brown algae)	79.88	11.88
Calculated $C_{27}H_{46}O_2$	80.59	11.44
Vitamin A from "Yatsume Unagi"	78.78	11.06 (ave.)

As shown above this result of the composition of vitamin A obtained from "Yatsume Unagi" was not agree with that of vitamin A in cod liver oil or in "Aonori". However, unfortunately the author could not study further and solve this difference of the percentage of carbon on account of its scanty sample.

Cholesterol separated during the extraction of Vitamin A from the oils was purified and showed melting point of 146.5–147°C.

Analysis :-	C %	H %
Sample I	83.95	11.66
II	83.19	11.51
Calculated $C_{26}H_{44}O$	83.87	11.83